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Current Directions in Autoimmunity

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Biologic and Gene Therapy of Autoimmune Disease

Volume Editor *C.G. Fathman*, Stanford, Calif.

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Current Directions in Autoimmunity

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Interleukin-10: Therapeutic Prospects in Rheumatoid Arthritis

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Introduction

The hallmark of interleukin-10 (IL-10) is its capacity to inhibit cytokine synthesis and downregulate a cellular immune response. Originally identified as a T helper 2 (Th2) cytokine [1], IL-10 has been increasingly recognized to play a much broader role in the regulation of immune function. IL-10 is a pleiotropic cytokine with diverse effects on a variety of cell types [reviewed in 2, 3]. It suppresses Th1-dependent cellular immune responses and supports Th2-dependent antibody-mediated immune responses. IL-10 inhibits the capacity of macrophages to synthesize proinflammatory cytokines and other mediators and serves as part of an endogenous feedback loop to restrain inflammatory stimuli.

Cytokine networks are pivotal in the pathogenesis of rheumatoid arthritis (RA). IL-10 is expressed by macrophages and T cells in the rheumatoid synovium [4]. While inhibitory, the levels of IL-10 are presumably inadequate to control joint inflammation. The inflammatory response is launched into overdrive by proinflammatory cytokines such as tumor necrosis factor (TNF)-α and IL-1 [5]. The vital role played by these proinflammatory cytokines in joint inflammation has been confirmed in clinical trials which show that TNF-α blockade and, to a lesser extent, IL-1 antagonism reduce the signs and symptoms of RA [6–11]. The administration of IL-10 has a similar therapeutic potential through its ability to suppress the production of proinflammatory cytokines.

Studies in both humans and mice provide insights into IL-10 functions. IL-10 shows relative species specificity. Human IL-10 has 71% sequence homology with mouse IL-10 [12, 13] and acts on both human and murine cells [3]. Mouse IL-10 demonstrates no activity on human cells [3]. Part of the human IL-10 sequence is highly homologous with an open-reading frame (BCRFI) in the Epstein-Barr virus (EBV) genome [13]. The viral IL-10 (vIL-10) shares with human and mouse IL-10 many of the same biological functions.

This chapter reviews the biology of IL-10 in the context of its possible therapeutic use in RA. Awareness of the diverse effects of IL-10 is crucial to properly design and interpret clinical studies. Also, individual differences in the genetic regulation of IL-10 may ultimately predict which patients may respond clinically to IL-10 interventions. Current clinical investigations of IL-10 may not only expand our therapeutic armamentarium for RA, but also shed new light on the complex mechanisms regulating aberrant immune responses.

Regulation of IL-10 Production

IL-10 is tightly regulated at the cellular level. Both murine and human monocytes produce high levels of IL-10 upon activation with the bacterial endotoxin lipopolysaccharide (LPS). LPS initially triggers a burst of TNF-α, IL-1, IL-6, and GM-CSF that reaches a peak at 3.5–7.5 h followed 20–48 h later by maximal synthesis of IL-10 [14]. LPS-stimulated IL-10 production depends on the synthesis of TNF-α and IL-1 [15, 16] as well as cognate interactions between monocytes and T cells [16]. TNF-α and IL-1 alone may also stimulate monocyte IL-10 production. The synthesis of IL-10 mRNA is inhibited by endogenous IL-10 production, creating an autoregulatory feedback loop [14].

Other factors regulate monocyte IL-10 production. TGF-β, IFN-α, and IFN-β enhance LPS-stimulated release of IL-10 [17–19]. IL-10 secretion may also be stimulated by ligating CD23, the low affinity IgE receptor [20]. IL-10 and IL-12 often are regulated in a reciprocal fashion. Ligation of the macrophage Fcγ receptor 1 (FcγRI) upregulates IL-10 production and inhibits IL-12 synthesis [21]. Histamine also stimulates IL-10 synthesis and suppresses IL-12 production [22]. Both IFN-γ and IL-4 downregulate LPS-induced IL-10 production [23, 24].

T cells produce IL-10, but in levels lower than monocyte/macrophages. The T cell-derived cytokines, including IL-10, can polarize the immune system toward a cellular or humoral response. The nomenclature for Th cell subsets is based on such cytokine patterns and includes the Th0, Th1 and Th2 subsets. In mice, Th1 cells produce IL-2, IFN-γ, and TNF-β, while Th2 cells generate IL-4, IL-5, IL-6 and IL-13 [25]. Th0, a precursor subset, produces IL-2, IFN-γ, IL-4 and IL-10. Murine Th2 and Th0 cells synthesize IL-10 [1, 25]. The

differentiation of the immune response is primarily determined by the relative amounts of IL-12 and IL-4. Monocyte-derived IL-12 differentiates the immune response to a Th1 cytokine pattern and cellular immunity. In comparison, IL-4 drives the immune system response to a Th2 cytokine profile which stimulates B cell-mediated humoral immunity, deactivates monocytes, and induces allergic responses [25]. Initial exposure of naive T cells to IL-4 polarizes the immune response to a Th2 phenotype. After about 3 weeks, previously committed Th1 cells restimulated with IL-4 respond by secreting IL-10, but not IL-4 or IFN-γ [26].

In man, T cell subsets with strictly Th1 and Th2 patterns of cytokine secretion are not readily distinguished by this classification system. Thus, the termminology Th1- and Th2-like cells has been used to describe overlapping phenotypes. Th0, Th1-like, and Th2-like cells have each been shown to produce IL-10. A high ratio of IL-10 to IFN-γ or IL-2 may determine the capacity of Th cells to inactivate an inflammatory response [27].

CD4 T cells secrete IL-10 when stimulated by anti-CD3 [27], anti-T cell receptor, anti-CD2 and anti-CD45 monoclonal antibodies, IL-1, phorbol myristate acetate, and forskolin, an activator of adenylate cyclase [28]. IL-4 has been shown to enhance IL-10 gene expression in murine Th2 cells without engagement of the T cell receptor [29]. IL-4 inhibits monocyte IL-10 production and stimulates T cell IL-10 secretion and, thereby, exerts paradoxical effects on the inflammatory response. IL-7 induces IL-10 mRNA in T cells [29]. The neuropeptides somatostatin and calcitonin gene-related protein stimulate murine antigen-specific Th0 and Th2 clones to synthesize IL-10 [30]. Other cell types that produce IL-10 include NK cells [31], B cells [32], EBV-transformed B cells [33], B cell lymphomas [34], mast cells [35], and keratinocytes [36].

The production of IL-10 may be potentially influenced by the rate of transcription, mRNA stability, efficiency of translation, and posttranslational events. While the synthesis of IL-10 appears to be controlled primarily at the transcriptional level [37], the 3'-untranslated region of the IL-10 mRNA contains multiple copies of the AU instability element. Thus, IL-10 mRNA is probably unstable in unactivated cells [34]. Individuals differ in the capacity of their peripheral blood cells to produce IL-10 in response to LPS. It has been estimated that 75% of these interindividual differences in LPS-stimulated IL-10 production are determined by heritability [39]. The innate capacity to generate IL-10 may be clinically relevant as suggested by the correlation between high IL-10 production and septic shock in bacteremic patients [40] and fatal outcome from meningococcal infection [39].

The regulation of IL-10 transcription has been investigated by analyzing the 5' flanking sequences of the human IL-10 gene. These studies identify a

TATA box at position -77 [41], several positive and negative regulatory elements [42], two highly polymorphic dinucleotide repeats (microsatellites) located 1.1 and 4.0 kb of the transcription initiation site [43, 44], and three polymorphisms at positions -1082, -819 and -592 [45]. The G to A nucleotide polymorphism at position -1082 is located within a putative binding site for the Ets transcription factors [41, 46]. The C to T nucleotide polymorphism at position -819 lies within a putative positive regulatory region. The C to A nucleotide polymorphism resides within a putative STAT-3 binding site and a negative regulatory region [41, 46]. The 5' flanking sequence of the IL-10 gene also contains binding sites for NFκB and REL proteins and STAT-1, as well as other cytokine response elements [42]. Preliminary evidence indicates that the IL-10 gene can specifically recognize members of the NFκB family of transcription factors [47].

The alleles at two microsatellite foci have been used to define haplotypes which are associated with different levels of LPS-induced IL-10 secretion by human blood cells [37]. In one study, individual haplotypes containing the allele IL-10.R2/IL-10.G14 were correlated with the highest IL-10 secretion; those defined by the allele IL-10.R3/IL-10.G7 had the lowest IL-10 secretion [37]. The -1082*A and *G alleles have been associated with low and high IL-10 production in vitro, respectively [45]. Furthermore, low levels of IL-10 production in vitro have been correlated with the ATA haplotype [46]. These data suggest that genotype may endow an individual with an innate capacity to secrete IL-10. Low IL-10-producing individuals might be at greater risk for RA or severe forms of this disease than high IL-10 producers. However, an analysis of 108 patients with early RA and 128 healthy controls did not reveal a significant correlation between disease and the presence of the -1082*A allele [48]. Similar negative results have been obtained in a study of 44 patients with Felty's syndrome, 117 patients with RA, and 295 healthy controls [49]. Eskdale et al. [50] have found that the IL-10.R2 allele occurs more frequently in controls than in Caucasians and African-Americans with RA. This finding shows paradoxically an association between high IL-10 production and disease likelihood.

Biological Properties of IL-10

IL-10 has diverse effects on many cells (table 1). In monocyte cultures, IL-10 inhibits LPS- or IFN-γ-induced stimulation of TNF-α, IL-1, IL-6, IL-8, GM-CSF and G-CSF [14]. The inhibition of TNF-α and GM-CSF production is over 90% [14]. vIL-10 similarly inhibits LPS-stimulated TNF-α and GM-CSF production [51]. The reduction in cytokine protein correlates

Table 1. Biological properties of IL-10

Inhibitory
Monocyte/macrophage
Inhibits production of TNF-α, IL-1, IL-6, IL-8, IL-10, IL-12, GM-CSF and G-CSF production
Inhibits generation of prostaglandins, NO, reactive oxygen species
Reduces expression of MHC class I and II molecules, CD80, CD86, ICAM-1 and formyl peptide receptor
Inhibits production of MIP-1α and MIP-1β
Inhibits production of soluble p55 and p75 TNF receptors
Neutrophils
Inhibits production of TNF-α, IL-1, IL-8
Reduces expression of formyl peptide receptor
T cells
Inhibits IL-2 production
Eosinophils
Inhibits TNF-α, GM-CSF and IL-8 production
Stimulatory
Monocyte/macrophage
Upregulates expression of FcγI
T cells
Protection from apoptosis
Chemoattractant for CD8 T cells
B cells
Stimulates proliferation, differentiation and Ig production
Increases IgG4 and IgE production
Protects against apoptosis
Mast cells
Stimulates proliferation

on Northern blots with a decrease in the steady-state amounts of the individual mRNAs [14]. The IL-10-induced reduction in LPS-stimulated cytokine mRNAs appears to occur at the posttranscriptional level in mouse [52] and at the transcriptional level in humans [53].

Fiorentino et al. [1] initially reported that murine IL-10, a product of Th2 cells, inhibited macrophage-dependent IFN-γ production by Th1 cells. Human and vIL-10 also inhibit IFN-γ production by Th cells [54]. This inhibitory effect on T cell cytokine synthesis results from reduced antigen-presenting capacity of monocytes. IL-10 downregulates class II MHC molecules on antigen-presenting cells (APCs) [14, 55] by inhibiting the transport of class II molecules from the cytoplasm to the cell membrane [56]. IL-10 also suppresses the costimulatory properties of monocytes by decreasing the expression of ICAM-1, CD80 and CD86 [57]. Monocyte-derived IL-12 is required early in the induction of Th1 responses. IL-12-driven T cell antigenic stimulation is negatively regulated by IL-10. IL-10 exerts this effect on IL-12 by decreasing the transcription of the p40 subunit of the IL-12 heterodimer without altering its mRNA stability [58].

IL-10 affects other aspects of monocyte activation and function. IL-10 inhibits LPS-and hyaluronan-induced expression of macrophage inflammatory protein (MIP)-1α and MIP-1β, two members of the C-C class of chemokines [59, 60]. IL-10 blocks conconavalin A-stimulated activity of prostaglandin H synthase-2/cyclooxygenase-2 and prostaglandin E_2-dependent synthesis of interstitial collagenase and gelatinase B (PGHS-2, COX-2) [61]. IL-10 markedly suppresses macrophage release of reactive oxygen intermediates [62] and nitric oxide (NO) [63]. High levels of NO are catalyzed by the inducible form of nitric oxide synthase (iNOS, NOS2), which is upregulated in circulating monocytes and synovial macrophages from patients with active RA [64]. The inflammatory response is dampened by cytokine inhibitors, such as IL-1 receptor antagonist (IL-1ra) and soluble TNF receptors. IL-10 increases soluble p75 TNF receptor levels in human monocyte cultures and diminishes monocyte cell surface p75 TNF receptors [65]. IL-10 by itself induces human monocytes to secrete low levels of soluble IL-1ra and significantly enhances LPS-stimulated production of IL-1ra [66]. IL-10 also upregulates FcγRI/CD64 expression in human monocytes [67], a potentially proinflammatory effect.

IL-10 acts directly on CD4 T cells to inhibit their production of IL-2 [68, 69]. Repetitive stimulation of murine CD4 T cell clones with IL-10 generates a T cell subset with low proliferative capacity and a unique cytokine profile [70]. These T cells, designated Tr1 cells, produce high levels of IL-10, very low levels of IL-2, and no detectable IL-4; the levels of IL-5, IFN-γ and TGF-β are similar to those of Th0 cell clones [70]. The IL-10 production by Tr1 cells probably explains their low proliferative capacity [70]. Tr1 cells suppress antigen-specific T cell responses in vitro and the development of inflammatory bowel disease in a SCID mouse model [70]. However, in mice, chronic IL-10 treatment fails to induce T cell tolerance in allogenic bone marrow recipients [71].

IL-10 also affects the behavior of CD8 T cells depending on their state of activation. Tumor and allospecific CD8 cytotoxic T cells pretreated with IL-10 show a reduction in autologous cytotoxic T lymphocyte-mediated, class I MHC-restricted tumor-specific lysis and allospecific cytotoxicity against EBV-

transformed lymphoblastoid cell lines [72]. This loss of cytotoxicity is associated with a decrease in the cell surface expression of class I MHC molecules, suggesting a novel mechanism of immune tolerance [72]. These effects are mediated through the capacity of IL-10 to inhibit APC costimulatory function [73]. T cells from patients with acute EBV-induced infectious mononucleosis rapidly die in vitro. However, these activated T cells are protected from undergoing apoptosis by treatment with human and vIL-10 [74]. Since IL-10 has similar effects on the survival of IL-2-dependent CD4 T cells [75], this cytokine may function in vivo to regulate T cell survival.

The effect of IL-10 on other cell types may have clinical implications. In cultures of neutrophils, IL-10 inhibits LPS-induced stimulation of TNF-α, IL-1, IL-8 and IL-12 production [76] and the surface expression of formyl peptide receptor [77]. Neutrophil surface expression of FcRγI is not downregulated by IL-10, unlike the situation for human monocytes [67]. IL-10 may modulate the inflammatory response in asthma and allergic disease by deactivating eosinophils [78]. In addition, IL-10 inhibits macrophage chemoattractant protein-1 production by activated gut epithelial cells [79], which may relate to mechanisms of bowel inflammation.

IL-10 stimulates the growth and differentiation of thymocytes, mast cells, and B cells [80–82]. IL-10 regulates humoral immunity and isotype switching. When human B cells are activated by IL-10 and an anti-CD40 monoclonal antibody they proliferate, differentiate into Ig-secreting cells, and switch to IgA, IgG1, and IgE isotypes [83]. IL-10 augments IgG4 production. On the other hand, IL-10 decreases the IL-4-induced switch to IgE, but enhances IgE production by B cells already switched to this isotype [83]. IL-10 prevents the spontaneous death of human splenic B cells by inducing the synthesis of bcl-2 protein [84].

IL-10 Receptor Signaling

The cDNA encoding a human IL-10R was originally isolated from a Burkitt lymphoma cell line and expressed in COS7 cells as a 90- to 110-kD protein [85]. The mouse IL-10R was similarly identified and expressed in a pro-B cell line (BaF3) that lacks IL-10 [86]. Based on sequence homology, the IL-10R was included with tissue factor, two subunits of the IFN-γ receptor (IFN-γR1 and IFN-γR2), and two subunits of the IFN-α receptor (IFN-αR1 and IFN-αR2) as a member of the type II cytokine receptor family. The original IL-10 receptor (IL-10R), now termed IL-10R1, primarily mediates high-affinity binding of IL-10. However, the observation that the single IL-10R chain did not reconstitute a functionally active receptor in fibroblasts led to

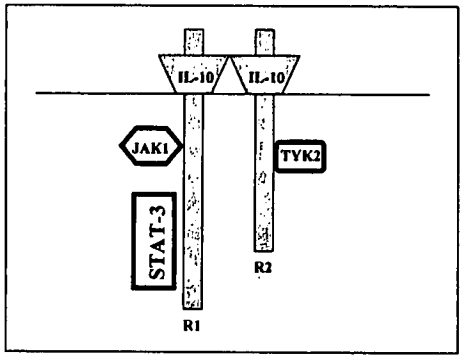

Fig. 1. IL-2R signaling depends on the JAK family of protein tyrosine kinases. The IL-10 homodimer binds to the IL-10R1 chain, leading to the formation of a complex with the IL-10R2 chain (CRF2-4). Jak1 binds to the IL-10R1 chain and Tyk2 interacts with the IL-10R2 chain. The Jaks phosphorylate specific tyrosine residues on the IL-10R1 chain, leading to STAT binding to the docking site on IL-10R1. STAT proteins then form dimers and translocate to the nucleus where they influence gene transcription.

the discovery of the second chain of the IL-10R CRF2–4. This chain, designated IL-10R2, joins the other members of the type II cytokine receptor family. It cooperates with the IL-10R1 chain for signaling [87, 88]. When the IL-10R1 chain interacts with IL-10 homodimer, it forms a complex with the IL-10R2 chain and initiates signal transduction [87, 89, 90].

The IL-10R utilizes the Janus family tyrosine kinase (JAK) signal transducers and activators of transcription (STAT) pathway [87, 88] (see fig. 1). Jak1 binds to the intracellular domain of the IL-10R1 chain [88]. Tyk2, another JAK family member, interacts with the IL-10R2 chain [88]. The JAKs phosphorylate specific tyrosine residues on the IL-10R1, which leads to the recruitment of STATs to their receptor docking site. IL-10 induces the activation of STAT-1, STAT-3 and STAT-5 [91]. STATs bind via their SH2 domains to the phosphorylated intracellular domain of the IL-2R1 chain. The STAT proteins are themselves activated by the JAKs. Tyrosine residues 427 and 477 are located within the intracellular domain of the mouse IL-10R1 and are required for IL-10 signaling [91, 92]. However, these two tyrosine residues are redundant in functional terms because mutant IL-10R1 chains expressing only one of these tyrosine residues behave similarly to the wild-type receptors [91, 92]. STATs can influence the regulation of many genes, including those for c-*fos*, E-selectin, c-*myc*, ICAM-1 and FcγRI [93].

IL-10 signaling presumably requires and additional signal beyond the recruitment of STAT-3. Studies have shown that STAT-3 is obligatory but not sufficient for IL-10 to inhibit LPS-induced TNF-α production [92]. Moreover, O'Farrell et al. [94] have shown that IL-10 inhibits TNF-α production in a Stat-3-independent manner. The molecular chain of events leading to other regulatory signals is still unclear. Riley et al. [92] have shown that IL-10 inhibition of LPS-induced TNF-α production requires additional serine residues in the carboxyl terminus of the IL-10R1 chain, suggesting that another protein may bind to the IL-10R1 chain and mediate IL-10-induced anti-inflammatory effects.

IL-10 influences several intracellular signaling pathways. IL-10 has been reported to reduce LPS-induced activation of $p56^{lyn}$ and Ras [95]. Ras, a small G protein, activates Raf-1, which binds to MEK1 and activates the mitogen-activated protein kinase (MAPK) cascade. MAPK pathways include the extracellular signal-related kinase (ERK), stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK), and $p38^{mapk}$ [93]. Some evidence has suggested that IL-10 does not effect LPS-induced activation of the MAPK kinases in human monocytes [96]. However, experiments in human dendritic cells show that TNF-α-induced phosphorylation of the MAPK cascade is significantly reduced by IL-10 [97]. Thus, in these experiments, IL-10 appears to block protein tyrosine kinase-mediated activation of ERK2, SAPK/JNK and $p38^{mapk}$. CD40 ligation by CD40 ligand (CD154) on T cells activates monocyte/macrophages to produce proinflammatory cytokines, which is similarly dependent on the generation of protein tyrosine kinase activity [98]. Suttles et al. [98] have shown using blood monocytes that CD40 signaling results in the phosphorylation of ERK1/2, but not SAPK/JNK or $p38^{mapk}$, and that preincubation with IL-10 reduces ERK1/2 kinase activity. Engagement of the IL-10R has also been shown to activate phosphatidylinositol 3-kinase and p70 S6 kinase, which stimulates monocyte proliferation without suppressing LPS-induced production of TNF-α or soluble TNF receptor [99]. Distinct signaling pathways may mediate the anti-inflammatory and proliferative effects of IL-10.

Animal Models

IL-10 antagonizes Th1 effector functions in vitro. The paradigm of a Th1-dependent immune reaction in vivo is delayed-type hypersensitivity (DTH). After recovery from *Leishmania major* infection, mice mount a highly polarized Th1 response after rechallenge with *L. major* antigen by injection of organisms into the footpads. Footpad swelling at 48 h after DTH induction is decreased by IL-10 treatment [100]. Neutralizing antibodies to IFN-γ fail to inhibit the

murine DTH response to *L. major* antigen, suggesting other cytokines mediate this response [100]. The DTH reaction induced by the injection of Th1 clones into mouse footpads is also inhibited by IL-10 treatment [101].

Susceptibility to certain protozoan, helminthic, and fungal infections is determined by innate immunity and the development of a strong Th1 response. Endogenous IL-10 may promote the survival of certain infectious agents by decreasing the induction of NO and microbicidal activity by macrophages and Th1-dependent synthesis of IFN-γ [102]. Treatment with anti-IL-10 antibody protects genetically susceptible DBA/2 mice from fatal *Candida albicans* infection, increases the frequency of IFN-γ-secreting splenic CD4+ T cells, and enhances macrophage capacity to produce NO [103]. In mice, high IL-10 doses exacerbate *C. albicans* [104] and *Listeria monocytogenes* [105] infection. In contrast, IL-10 may not be a factor in the outcome of murine leishmaniasis [106].

The control of infection involves a regulatory loop between IL-10 and IL-12. IL-10 treatment inhibits IL-12 production in IL-10-deficient mice infected with *Toxoplasma gondii* and protects against lethality in these animals from the overproduction of IL-12, TNF-α and IFN-γ [107]. In murine candidiasis, IL-12 induces IL-10 production by T cells and neutrophils [103, 108]. IL-12 genetically deficient mice infected with *C. albicans* show defective CD4+ Th1 cellular responses and attenuated macrophage production of IL-10 [109]. In these mice, short-term, low-dose IL-10 treatment expands the protective CD4+ T cell repertoire [109]. IL-10 may afford this protection by inhibiting CD80 and CD86 expression on APCs, preventing the expansion of IL-4-producing CD4+ T cells, and skewing the immune responses to a Th1 phenotype [109]. Paradoxically, high IL-10 doses can exacerbate *C. albicans* infection in mice.

The importance of IL-10 in immune function has been investigated in IL-10 transgenic mice. In some transgenic mice, the IL-10 gene is driven by a T cell-specific promoter that regulates T cell-specific expression of IL-10 from the earliest stages of T cell stimulation. T cell-dependent oversecretion of IL-10 in this case inhibits IFN-γ production without stimulating IL-4 synthesis or antibody class switching [110]. IL-10 transgenic T cells fail in some instances to mediate Th1-dependent pathologic responses. Transfer of CD4+ $CD45RB^{high}$ splenic T cells from normal mice to SCID or $RAG\text{-}2^{-/-}$ immune-deficient recipients produces a Th1-mediated inflammatory bowel disease. However, this experimental disease is not transferred by CD4+ $CD45RB^{high}$ T cells from the IL-10 transgenic mice [110]. The transgenic mice also develop larger tumors after injection of tumor cells than their nontransgenic littermates [110]. Not all Th-1 immune responses are defective in the IL-10 transgenics. These mice still develop a protective Th1 response

to *L. major* infection [110]. However, in another transgenic model where the T cells are engineered to overproduce IL-10, the mice show impaired control of mycobacterial infection [111].

IL-10 has also been overproduced in APCs which express a human IL-10 cDNA under the control of a mouse class II MHC Ea promoter. Splenic macrophages from the APC IL-10 transgenics and their wild-type littermates do not differ in the expression of MHC molecules, ICAM-1 and CD86 molecules [112]. These IL-10 transgenics are characterized by certain defects in Th1- and Th2-type responses and show increased susceptibility to infection with *L. monocytogenes* and *L. major* [112]. Leishmaniasis infection progresses in these IL-10 transgenic mice despite protective Th1-like response to *L. major*, implying that IL-10 primarily acts to inhibit macrophage microbicidal effector functions. Treatment with a neutralizing anti-IL-10 antibody reverses the impaired host responses in these transgenic mice [112].

IL-10 is an important mediator in shock. Staphylococcal enterotoxin B, a superantigen, triggers IL-2 and TNF-α release into the circulation of mice, leading to shock. Staphylococcal enterotoxin B also promotes release of IL-10, which limits the deleterious effects of shock in this mouse model [113]. In LPS-challenged mice, pretreatment with IL-10 markedly reduces the amount of TNF-α released into the circulation and completely prevents mortality from shock [114]. However, exogenous IL-10 fails to decrease mortality in the more clinically relevant cecal ligation and puncture model of sepsis [115].

The biological effects of IL-10 have been investigated in experimental animal models of autoimmunity. Immunization with myelin antigens induces experimental allergic encephalomyelitis (EAE), a Th1-mediated disease characterized by weight loss, weakness of the tail and hind limbs, and ascending paralysis. Clinical recovery from EAE occurs with increased CNS expression of IL-10 [116]. Systemic administration of IL-10 during the initiation phase of EAE markedly suppresses the clinical manifestations of disease [117]. Mice expressing the human IL-10 transgene under the control of the MHC class II promoter are relatively resistant to EAE induction [118]. This inhibitory effect is probably related to the inability of the myelin-reactive Th1 cells to support effector cell function [118].

Systemic therapy with human IL-10 prevents the onset of diabetes in nonobese diabetic (NOD) mice [119]. IL-10-treated NOD mice show less insulitis than untreated mice and their β cells secrete normal insulin levels [119]. A contrasting IL-10 effect on diabetes has been observed in transgenic mice engineered to express IL-10 in pancreatic β cells. In this case, local IL-10 production accelerates the onset of diabetes in the NOD mouse and increases its prevalence [120]. Transgenic mice expressing lymphocytic choriomeningitis virus (LCMV) antigen in the pancreas develop diabetes after LCMV infection

[121]. IL-10 and LCMV double transgenic mice develop diabetes earlier than LCMV single transgenics. Thus, complex interactions, both systemic and local, determine the ultimate outcome of IL-10 treatment in the NOD mouse.

IL-10-deficient mice develop chronic colitis mediated by IFN-γ-secreting CD4+ T cells [122]. Only proximal bowel inflammation develops in transgenic mice kept under pathogen-free conditions, suggesting that the full-blown disease depends on antigen-specific stimulation by bacteria [122]. Treatment of IL-10-deficient mice with anti-IL-12 antibody prevents colitis in both young and adult mice, while anti-IFN-γ antibody treatment has only a minimal effect on established disease in the adult mice [123]. IL-12 presumably sustains the Th1 response during the chronic phase of this illness [123].

Transgenic mice expressing IL-10 under the control of a human salivary gland amylase promoter develop an exocrinopathy resembling Sjögren's syndrome [124]. The lacrimal and salivary glands in these mice are infiltrated with CD4+ T cells [124]. The glandular cells express high levels of Fas and are undergoing a high rate of apoptosis [124]. The infiltrating CD4+ T cells are polyclonal and express Fas ligand (FasL) [124], suggesting that glandular tissue destruction may result from nonspecific Fas/FasL interactions. IL-10 apparently can function either as an immunosuppressive or immunostimulatory cytokine depending on the model.

Evidence from animal models shows that IL-10 plays an important role in arthritis. Similar to RA in humans, type II collagen arthritis (CIA) in the mouse is characterized by synovial proliferation, the accumulation of leukocytes in the synovial tissue, and destruction of articular cartilage and subchondral bone. The development of arthritis is accompanied by expression of IL-10 in synovial macrophage-like and fibroblast-like cells as well as chondrocytes [125]. Mice treated with a neutralizing anti-IL-10 antibody show an acceleration of arthritis [125]. On the other hand, IL-10 treatment suppresses both early and established CIA [126, 127]. IL-10 administration reduces histologic signs of inflammation, TNF-α and IL-1 mRNA levels in synovial tissue, and cartilage and bone destruction [126, 127]. High doses of IL-12 boost IL-10 production in murine CIA and attenuate joint inflammation [128], providing further evidence that IL-10 is important in the pathogenesis of CIA. However, streptococcal cell wall-induced arthritis develops independent of IL-10 production despite the participation of T cells and macrophages [129].

Gene delivery of IL-10 has been investigated as a possible therapeutic strategy for arthritis. The overexpression of IL-10 in close proximity to a joint facilitates the maintenance of inhibitory concentrations at the desired site with potentially less systemic exposure. vIL-10 lacks immunostimulatory activity and thus may have therapeutic advantages over human or mouse IL-10. A nonreplicative adenovirus vector has been engineered to express vIL-10

(Adv-vIL-10) under the control of a specific promoter. Intravenous administration of Adv-vIL-10 delays the onset of murine CIA and reduces its severity [130, 131] in association with decreased splenic T cell responses to type II collagen [130]. Synovial tissue from successfully treated mice shows diminished inflammation [131] and lower levels of IL-2 and IL-1 mRNAs compared to control mice [130]. While gene delivery of vIL-10 suppresses the initiation of CIA, this approach fails to control established disease [130]. Moreover, other investigators find that intravenous Adv-vIL-10 injections have no effect on CIA [132]. In CIA, administration of Adv-vIL-10 by the intra-articular route exacerbates inflammation in the injected joint, but suppresses arthritis in the noninjected joints [130]. Periarticular Adv-vIL-10 injection protects the joint from CIA in the proximity of the injection as well as reduces inflammation in the noninjected joints [132].

Gene therapy has also been examined in SCID mice engrafted subcutaneously with cartilage and synovial fibroblasts which have been transduced with a retroviral vector expressing the murine IL-10 or vIL-10 gene [133]. Both forms of IL-10 inhibit fibroblast invasion of cartilage, but have no effect on perichondrocytic cartilage degradation [133]. This inhibitory effect of IL-10 on fibroblast invasiveness has also been observed in a SCID mouse engrafted with human rheumatoid synovium and homologous cartilage [134]. Systemic vIL-10 delivery abrogated cartilage invasion by synovial tissue, but did not diminish the T cell infiltrate or decrease the IL-1, IL-6, TNF-α or IL-8 mRNA levels in synovial tissue [134].

IL-10 in RA

IL-10 is synthesized by macrophages and T cells in rheumatoid synovial tissue where it acts in concert with other factors to regulate the inflammatory response [5]. The immunoregulatory role of IL-10 has been investigated in synovial tissue cultures which spontaneously produce TNF-α, IL-1, IL-6 GM-CSF and IL-8 as well as IL-10 [5, 135]. These cell preparations are a heterogenous population of synovial cells and immune cells. The addition of TNF-α and IL-1 stimulates IL-10 production in culture [5]. Synovial cell cultures incubated with anti-IL-10 antibody show higher levels of TNF-α and IL-1, indicating that endogeneous sources of IL-10 are suppressing the production of these proinflammatory cytokines [5]. However, IL-10 blockade has little or no effect on IL-6 or IL-8 production [5]. On the other hand, exogenous IL-10 markedly reduces TNF-α and IL-1 levels in synovial cell cultures without significantly altering IL-6, IL-8 [5] or IL-1ra levels [135]. In other experiments, exogenous IL-10 has shown modest inhibitory effects on IL-6 [135] and IL-8 production [136]. These results provide a rationale for using systemic IL-10 therapy as a means to reduce joint inflammation.

The effects of IL-10 may depend on contact with other cell types and mediators in the synovial microenvironment. This fact must be kept in mind when interpreting experiments using partially purified synovial cells. The addition of IL-10 to cultures of synovial fibroblasts does not affect IL-6 and GM-CSF production [137]. In contrast, similar experiments using a macrophage-like, CD14-enriched population of synovial cells find that IL-10 inhibits IL-6 production in culture [137]. IL-10 has been shown to inhibit synovial cell production of IL-1, IL-6, IL-8, G-CSF and GM-CSF production and IFN-γ-induced expression of HLA-DR, ICAM-1 and VCAM-1 in synovial cell cultures containing about 10% CD14+ macrophage-like cells [138]. Most of these observed IL-10 effects are probably dependent on macrophage-like synovial cells.

IL-10 and to a lesser extent IL-2 has the ability to stimulate antibody production in cocultures of synoviocytes with purified human tonsillar B cells [139]. Moreover, synovial fluid B cells produce increased amounts of IgM rheumatoid factor when they are cocultured with synoviocytes in vitro [140]. IL-10 also stimulates IgM rheumatoid factor production in this culture system [140]. These results suggest that upregulated expression of IL-10 may explain in part the accumulation of IgM rheumatoid factor-secreting B cells in the synovial microenvironment.

Synovial T cells represent an important source of IL-10. T cell clones isolated from the rheumatoid joint are predominately Th1-like [141]. Most of the stimulated CD4+ T cell clones produce IFN-γ and IL-10. In agreement with these data, immunochemical analysis of synovial tissue shows 0.6% IFN-γ- and 1.5% IL-10-positive CD4+ T cells [141]. Stimulation of synovial CD4+ T cells with phorbal myristate acetate plus calcium ionophore also shows that most of the IL-10-secreting CD4+ T cells produce IFN-γ [142].

IL-10 mRNA and protein are detectable in synovial fluid from patients with RA [143, 144]. The main source of IL-10 in synovial fluid is the mononuclear cell, which spontaneously produces this cytokine in culture [144, 145]. In mononuclear cell cultures, exogenous IL-10 suppresses the production of TNF-α, IL-1 and GM-CSF, reduces the expression of HLA-DR molecules, enhances the expression of CD16 and CD64, and decreases spontaneous cell proliferation [144, 145]. Synovial fluid mononuclear cells stimulated with IL-10 in culture show increased expression of surface and soluble p75 recetor in culture [146].

IL-10 directly stimulates normal cartilage to synthesize proteoglycans [145]. Conditional media from antigen-stimulated synovial fluid mononu-

clear cells inhibit proteoglycan synthesis by cultured cartilage explants [145]. The cartilage degradation is largely dependent on the presence of IL-1 and TNF-α. IL-10 treatment of synovial fluid mononuclear cells reverses the inhibition of proteoglycan synthesis induced by the conditioned media [145].

Elevated IL-10 serum levels have been reported in patients with RA [143]. Peripheral blood mononuclear cells spontaneously produce IL-10 in vitro [147], and probably represent the main source of circulating IL-10. Although serum levels of IL-10 do not correlate with clinical measures of disease activity in RA, they do significantly correlate with serum rheumatoid factor titers and the amount of spontaneous IgM production in vitro [143]. IgM production in peripheral blood is dependent on endogenous IL-10 [148]. Blood monocytes incubated in culture with IL-10 show decreased production of TNF-α, IL-1 [145], and IL-6 [135] and increased expression of surface p55 and p75 TNF receptor and soluble p75 TNF receptor [146]. Both endogenous and exogenous TNF-α have been shown to mediate IL-10 release in vivo [149].

Clinical Studies of IL-10

The immunomodulatory effects and safety of systemic IL-10 administration has been studied in 17 healthy subjects randomized to receive a single intravenous bolus injection of 1, 10 or 25 μg/kg recombinant human IL-10 or placebo [150]. IL-10 administration was associated with a transient neutrophilia, monocytosis, and lymphopenia, and a significant reduction in both the CD4 and CD8 T cell subsets [150]. The two highest doses of IL-10 produced up to a 50% decrease in mitogen-induced proliferation of peripheral blood mononuclear cells [150]. IL-10 also inhibited TNF-α and IL-1 production by whole blood stimulated ex vivo with endotoxin, but had no effect on soluble p55 TNF receptors or IL-1ra [150]. There were no significant adverse effects in this study.

A phase I dose-escalating, double-blind, placebo-controlled trial has been conducted in 72 patients with active RA [151]. In this trial, subjects were randomly allocated to receive daily subcutaneous injections for 4 weeks of either 0.5, 1.0, 5, 10 or 20 μg/kg of recombinant human IL-10 or placebo. The recombinant IL-10 injections were well tolerated during the study. There was a reduction in platelet counts in subjects treated with the highest doses of IL-10, including 4 subjects receiving the 20 μg/kg dose who had a platelet count decrease below 100,000/mm^3. A trend towards clinical improvement was observed in the group receiving 5 μg/kg of recombinant IL-10. The highest

dose groups had a significant increase in soluble p55 TNF and p75 receptor and IL-1ra levels.

Further trials have been performed in patients with RA using IL-10 as monotherapy or combined with methotrexate. A preliminary analysis has been completed for a phase II trial of IL-10 in patients with active RA receiving methotrexate therapy. The results suggest that subcutaneous injections of IL-10 as well tolerated and produce a favorable clinical response. A larger phase II/III trial of recombinant IL-10 therapy in patients with active RA is now in progress.

Conclusion

A growing understanding of the pathogenesis of joint inflammation has enabled rational targeting of specific pathways to achieve therapeutic goals. IL-10 interfaces with the cytokine cascade at several key points to maintain control over the inflammatory response, making it an attractive anti-inflammatory agent. Studies in animal models confirm the anti-inflammatory properties of IL-10 and reveal its complex interplay with other cytokines in the regulation of immune function and host defense. Clinical trials will ultimately provide the answer of whether the results in animal models can be extrapolated to a complex human disease such as RA.

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